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Desumoylation of homeodomain-interacting protein kinase 2 (HIPK2) through the cytoplasmic-nuclear shuttling of the SUMO-specific protease SENP1

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Abstract The modification of homeodomain-interacting protein kinase 2 (HIPK2) by small ubiquitin-like modifier 1 (SUMO-1) plays an important role in its targeting into the promyelocytic leukemia body, as well as in its differential interaction with binding partner, but the desumoylation of HIPK2 by SUMO-specific proteases is largely unknown. In this study, we show that HIPK2 is a desumoylation target for the SUMO-specific protease SENP1 that shuttles between the cytoplasm and the nucleus. Mutation analyses reveal that SENP1 contains the nuclear export sequence (NES) within the extreme carboxyl-terminal region, and SENP1 is exported to the cytoplasm in a NES-dependent manner. Sumoylated HIPK2 are deconjugated by SENP1 both in vitro and in cultured cells, and the desumoylation is enhanced either by the forced translocation of SENP1 into the nucleus or by the SENP1 NES mutant. Concomitantly, desumoylation induces dissociation of HIPK2 from nuclear bodies. These results demonstrate that HIPK2 is a target for SENP1 desumoylation, and suggest that the desumoylation of HIPK2 may be regulated by the cytoplasmic-nuclear shuttling of SENP1.

Keywords: Homeodomain-interacting protein kinase 2; SENP1; Small ubiquitin-like modifier; Nuclear export

1. Introduction

The homeodomain-interacting protein kinase 2 (HIPK2) both interacts with and phosphorylates a variety of transcription factors, including homeoproteins, the Groucho corepressor, CtBP1, Myb, and p53, all of which represent critical regulators of cell fate decisions and apoptosis during development [1–7]. As such, studies of the HIPK2 knock-out mice have revealed that HIPK2 is a critical component of transcriptional machinery that controls sensory neuron survival [8,9]. HIPK2 is the first protein kinase shown to be modified by small ubiquitin-like modifier 1 (SUMO-1) [10]. The sumoylation of HIPK2 occurs at multiple sites, and affects its interactions with binding partners, as well as its stability and recruitment into the promyelocytic leukemia (PML) body [11–13]. To date, however, little is known about desumoylation of HIPK2.

A growing number of proteins have been designated as targets of the SUMO-modification system [14,15]. The SUMO conjugation pathway has been shown to employ a series of enzymes that exhibit sequence similarity to analogous enzymes within the ubiquitin pathway, and also utilizes similar biochemical mechanisms [16]. Like ubiquitin system, SUMO-modifications appear to be dynamic and reversible. As predicted, the first SUMO-specific protease, Ulp1, has been identified in yeast recently [17]. Thereafter, many SUMO-specific proteases are now known in mammals, which share sequence similarity in their C-terminal domains with the catalytic Ulp domain of yeast Ulp1 [16,18,19]. However, desumoylation targets for SUMO-specific protease are largely unknown and begun to be identified. One of the interesting features of the SUMO-specific proteases is their subcellular localization. Yeast Ulp1 and Ulp2 are detected at the nuclear pore and nucleoplasm, respectively [20,21]. The mammalian SENP1 is localized mainly in the nucleus [18], and the nuclear localization sequence (NLS) of SENP1 was recently characterized [22]. SENP3 is localized in the nucleolus whereas SUSP1/SENP6 is localized in the cytoplasm [23,24]. In the case of SENP2, the largest SENP2/Axam is associated with the nucleoplasmic face of nuclear pore [25]. The shorter isoforms Axam2 and SuPr-1, which lack the N-terminal NPC targeting sequence, were detected in the cytoplasm and the PML bodies, respectively [26,27]. Therefore, the distinct subcellular localizations of these proteins may target them toward discrete sets of substrates, thereby allowing for the selective deconjugation of SUMO-modified proteins [14]. However, domains and factors regulating subcellular localization of SUMO-specific proteases are less understood.

In this report, we have demonstrated that HIPK2 is a desumoylation target for SENP1. Furthermore, we have shown that, depending on different cell types, SENP1 can be localized either in the cytoplasm or in the nucleus, and that SENP1 is exported to the cytoplasm through the nuclear export sequence

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Abbreviations: HIPK2, Homeodomain-interacting protein kinase 2; SUMO, small ubiquitin-like modifier; Myc-tag, Myc-epitope tag; PML, promyelocytic leukemia; GST, glutathione *S*-transferase; DAPI, 4′,6-Diamidino-2-phenylindole; LMB, Leptomycin B; NES, nuclear export sequence; NLS, nuclear localization sequence; NB, nuclear body; GFP, green fluorescent protein

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(NES) which maps to the carboxyl-terminal end of the protein. Our results provide a potential mechanism by which desumoylation of HIPK2 can be regulated through the cytoplasmic-nuclear shuttling of SENP1.

2. Materials and methods

2.1. Cell culture, transfection and Western blot

U2OS and CV-1 cells were grown in Dulbecco's modified Eagle's medium, supplemented with 10% fetal bovine serum. For immunoblot analysis, CV-1 cells were seeded onto six-well plates, and DNA transfection was carried out using the *N*,*N*-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid-buffered saline version of the calcium phosphate method. Western blots were performed with indicated antibodies as described previously [10].

2.2. Plasmid construction and site-directed mutagenesis

Myc-HIPK1, Myc-HIPK2, and EGFP-SUMO-1 were described previously [1,10]. The Myc-SENP1 plasmid was constructed by the insertion of cDNA, which is amplified by PCR using template DNAs from the human placenta cDNA library (Clontech), into the *Eco*RI and *Xba*I sites of pCS3MT. The Myc-SENP1(C603A) and EGFP-SENP1 NES-M mutants were generated using the QuickChange mutagenesis kit (Stratagene) according to the manufacturer's recommendations. All mutations were verified by DNA sequencing. EGFP-2x SENP1 NES was constructed by the insertion of double strand oligonucleotides (5′-AATTCATGGTCTGGGAGATCCTCCACCGAAAACTCCTC-GTCGACATGGTCTGGGAGATCCTCCACCGAAAACTCTTGTG-AG-3′) encoding two copies of SENP1 NES (MVWEILHRKLL) into the *Eco*RI and *Sal*I sites of pEGFP-C2 (Clontech).

2.3. Generation of transgenic fly lines

The DNA fragments encoding wild-type SENP1 were excised from EGFP-SENP1, and were introduced into the *Eco*RI/*Xba*I sites of the P-element vector pUAST [28]. The transgenic lines harboring *UAS-GFP-SENP1* were established using standard procedures as described [29]. Five different transgenic lines for wild-type SENP1 were established, and at least two distinct transgenic lines were crossed with the *fkh-GAL4* driver line [30] to investigate the potential variations of SENP localizations in vivo. Fly growth and cross of transgenic lines were conducted at 25 °C according to the standard procedures.

2.4. Immunocytochemistry

CV-1 cells were grown on coverslips and transfected with the Myc-HIPK2 and GFP-SENP1 expression vectors. Thirty-six hours after transfection, cells were fixed for 15 min with 10% formaldehyde at room temperature, then incubated with a solution containing 1× PBS and 0.5% Triton X-100. All subsequent procedures were done as described previously [10]. In order to obtain the images of GFP-SENP1 in the salivary gland, the salivary gland and its associated tissues were isolated from third instar larvae, followed by fixation and staining using 4′,6-diamidino-2-phenylindole (DAPI). The GFP-SENP1 and DAPI images were acquired with a confocal laser scanning microscope, a Zeiss LSM510. The acquired images were then processed with Adobe Photoshop. For time-lapse imaging of living cells, CV-1 cells were transfected with GFP-SENP1. After 24 h transfection, cells were treated with Leptomycin B, and images of cells were taken at different time points.

2.5. In vitro desumoylation

GST-SENP1 fusion proteins were expressed in *Escherichia coli* and purified with a glutathione–Sepharose column according to manufacturer's protocol. For the isolation of the SUMO-modified HIPK2 as a substrate of the in vitro reaction, cells were cotransfected with Myc-HIPK2 and GFP-SUMO-1 expression vectors. Subsequently, Myc-HIPK2 proteins (both modified and unmodified-HIPK2) were immunoprecipitated with anti-Myc antibodies and diluted in the reaction buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 1 mM dithiothreitol). Increasing amounts of GST-SENP1 (1 or 2 μg) were added and the mixture was incubated at 37 °C for 1 h. The desumoylation of the HIPK2-SUMO-1 conjugates was detected by Western blotting using anti-Myc antibodies.

3. Results

3.1. Nuclear or cytoplasmic localization of SENP1

HIPK2 conjugation by SUMO-1 plays an important role in the localization of HIPK2 [10,13], as well as its differential interactions with binding partners [11]. In order to gain insight

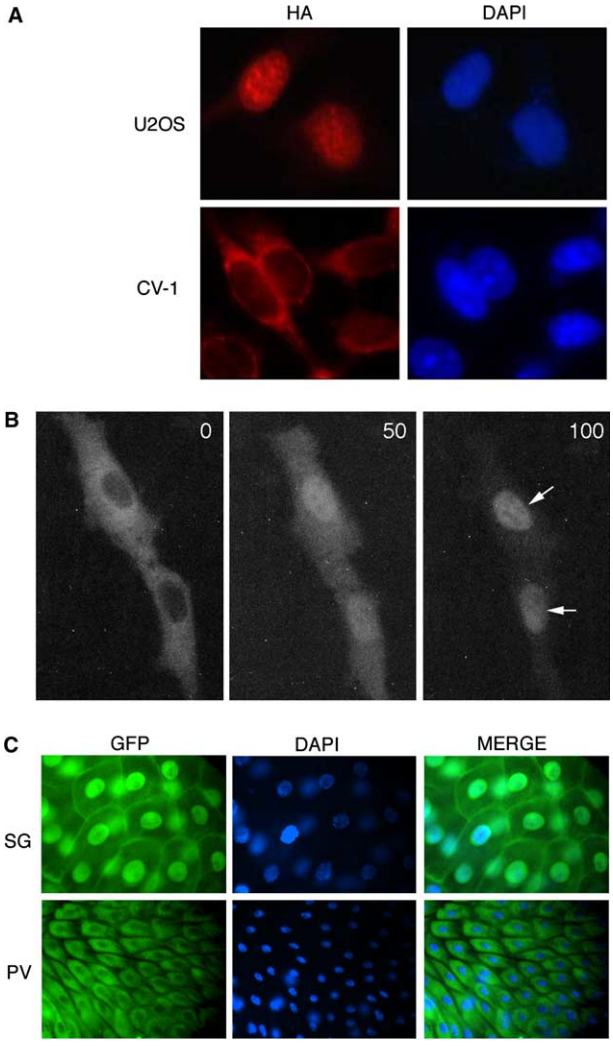

Fig. 1. Nuclear or cytoplasmic localization of SENP1. (A) Plasmids encoding HA-tagged human SENP1 were transfected into U2OS and CV-1 cells. The cells were then fixed and incubated with anti-HA antibody, and followed by detection with rhodamine-conjugated anti-mouse secondary antibody. The nuclear or cytoplasmic localization of SENP1 was assessed via comparison with DAPI staining images. (B) The GFP-SENP1 plasmids were transfected into CV-1 cells. Thirty-six hours after transfection, LMB was applied to the cells. A typical image was obtained with a confocal laser scanning microscope, at 50 and 100 min after LMB treatment. The nuclear relocalizations of GFP-SENP1 are shown with arrows. (C) The *UAS-GFP-SENP1* transgenic lines were crossed with the *fkh-GAL4* driver lines. A typical image of GFP-SENP1 was acquired from the salivary gland (SG) and the proventriculus (PV) of the *Drosophila* third instar larvae. The nuclei of the cells were decorated with DAPI staining.

into HIPK2 regulation by desumoylation, we attempted to characterize the enzyme responsible for the desumoylation of HIPK2. Among known SUMO-specific proteases, SENP1 was chosen to study because it seems to be the most effective with regard to the removal of SUMO-1 moiety from sumoylated HIPK2 (data not shown). During this study, we found that SENP1 localized in either the cytoplasm or nucleus, depending on the cell type (Fig. 1). HA-tagged human SENP1 was found to localize mainly in the cytoplasm of the CV-1 cells, whereas it localized in the nucleus of the U2OS cells (Fig. 1A). These results indicated that the subcellular localization of SENP1 could be changed, depending on the cellular context. In order to determine whether SENP1 can be exported from the nucleus, CV-1 cells were transfected with GFP-SENP1. After 24 h transfection, cells were treated with Leptomycin B (LMB), an inhibitor of the nuclear export factor CRM1, and green fluorescent protein (GFP) signals from transfected living cells were monitored. All of the visible GFP-SENP1 was retained in the nucleus 100 min after the application of LMB (Fig. 1B). This result indicated that the LMB blocked nuclear export of GFP-SENP1, and suggests that SENP1 shuttles between the cytoplasm and the nucleus. In order to further explore the localization patterns of SENP1 in vivo, we generated transgenic fly lines harboring a GFP-SENP1 transgene. The transgenic lines were crossed with the *fkh-GAL4* driver lines in order to induce expression of GFP-SENP1 in the salivary gland and the gut primordia of the *Drosophila* third-instar larvae [30]. In the salivary gland, GFP-SENP1 localized to both the nucleus and the cytoplasm including the plasma membrane, whereas GFP-SENP1 existed predominantly in the cytoplasms of the cells comprising the proventriculus, a glandular portion of the foregut (Fig. 1C). These results clearly indicate that GFP-SENP1 can be localized in either the nucleus or cytoplasm, depending on the cellular context and can shuttle between the nucleus and the cytoplasm (or plasma membrane) in vivo.

3.2. Characterization of NES of SENP1

The finding of the relocalization of cytoplasmic GFP-SENP1 into the nucleus upon treatment of LMB compelled

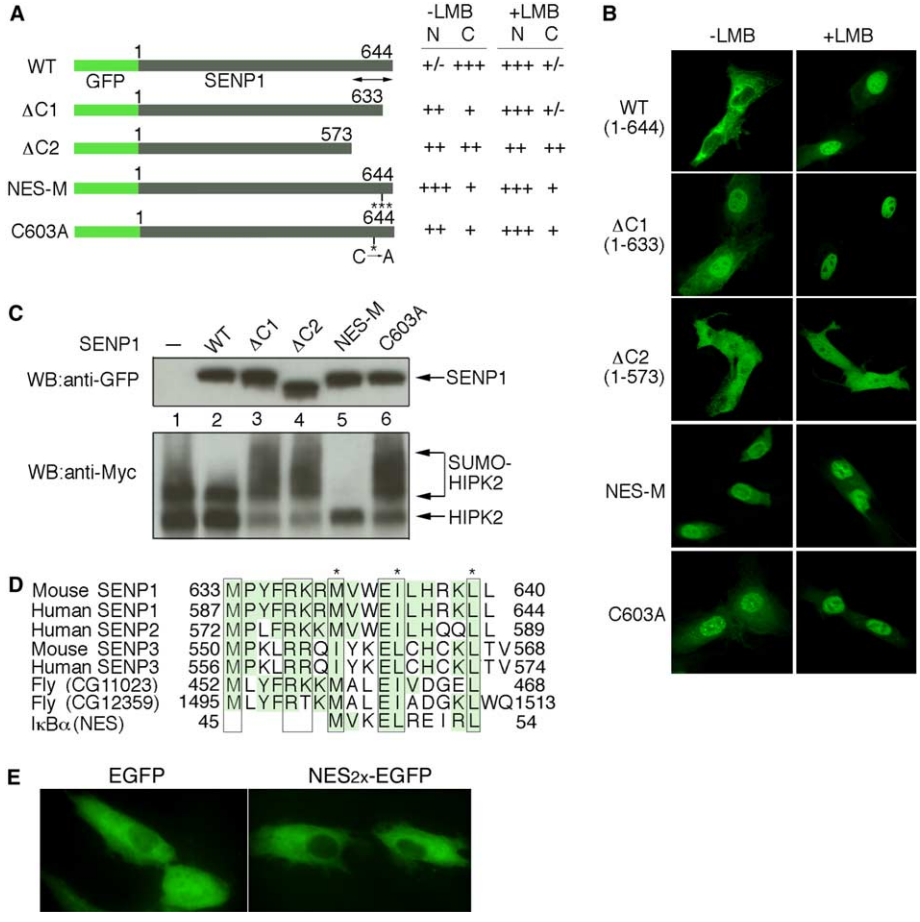

Fig. 2. Characterization of the NES motif of SENP1. (A) Schematics of the various SENP1 deletion and point mutants. Their localizations both before and after LMB treatment are summarized to the right of the schematics. Intensity of GFP signal detected either in the nucleus (N) or in the cytoplasm (C) is marked as follows: +/−, barely detectable; +, weak; ++, medium, +++, strong. (B) The GFP-SENP1 mutants were transfected into CV-1 cells, and typical images were obtained for each mutant both before and after the application of LMB treatment. (C) The GFP-SENP1 mutant plasmids were transfected into CV-1 cells along with Myc-HIPK2 and His-SUMO-1 expression plasmids. The levels of SENP1 mutant expression, as well as the extent of HIPK2 sumoylation, were determined with Western blotting using anti-GFP and anti-Myc antibodies, respectively. (D) The amino acids corresponding to SENP1 NES were aligned with those of the related SUMO-specific proteases as indicated in the figure. The conserved amino acids are boxed and the amino acids mutated in the SENP1 NES-M are marked as asterisks. (E) The EGFP-C2 and 2xNES-EGFP plasmid encoding EGFP and EGFP-SENP1 NES fusion protein, respectively, were transfected into CV-1 cells, and images were obtained 36 h after transfection.

us to attempt to map the motif responsible for SENP1 nuclear export. We constructed and transfected a variety of GFP-SENP1 deletion mutants into CV-1 cells, and examined the localizations of SENP1 mutants (Fig. 2A). The deletion of the C-terminal portion to aa 634 induced the nuclear localization of SENP1 (Fig. 2B, ΔC1), suggesting that the motif for SENP1 export is located in this region. Further deletion to aa 574 resulted in the diffused localization of SENP1 both to the nucleus and the cytoplasm (Fig. 2B, ΔC2), and this distribution persisted unchanged after treatment of LMB. This result suggests that there might be a motif for the nuclear localization of SENP1 in the region from aa 574 to 633 (Fig. 2B). The amino acid alignment of C-terminal portion of SENP family proteins indicated that some specific amino acids are particularly well-conserved between various SENP family proteins, and some portion of these sequences exhibited a profound similarity with the NES motif of *IκB* (Fig. 2D). In order to confirm the function of the NES motif, the conserved amino acids of M634, I638 and the L643 of SENP1 were substituted with alanines. The resulting SENP1 NES-M mutant was preferentially localized to the nucleus of the CV-1 cells, irrespective of LMB treatment (Fig. 2B). This finding strongly suggests that the NES motif is located within the extreme C-terminus, and also that SENP1 might be exported via this motif. This NES motif appears to be rather well-conserved between *Drosophila*, mouse, and human SENP1, and between SENP1, SENP2, and SENP3 (Fig. 2D), suggesting that nuclear export is vitally important for the regulation of several members of the SUMO-specific proteases family as well as for the regulation of SENP1. The conserved SENP1 NES (MVWEILHRKLL) was sufficient to export GFP when two copies of the NES was fused to the C-terminal EGFP (Fig. 2E). The nuclear localization of the catalytic mutant SENP1(C603A) was consistent with the finding reported previously [22]. The desumoylation activities of the SENP1 mutants were analyzed with Western blotting using anti-Myc antibodies (Fig. 2C). The NES-M SENP1 mutant exhibited a much more pronounced desumoylation activity toward the HIPK2-SUMO conjugates than did the wild-type SENP1 (Fig. 2C, lane 5), which was consistent with the results seen with the application of LMB treatment (see below, Fig. 4D). The ΔC1 SENP1 mutant was not found to remove the SUMO-1 moiety from the HIPK2-SUMO conjugates, despite its nuclear localization. This suggests that the residues for SENP1 catalytic activity might be deleted in this mutant. When all of these results are considered, it would appear that both the nuclear localization signal and the NES of SENP1 might function to determine the location of SENP1 in cultured cells.

3.3. Desumoylation of HIPK2 by SENP1

As HIPK2 sumoylation affects its localization within the nucleus, we initially attempted to visualize HIPK2 localization in the presence of SENP1. CV-1 cells were transfected with the plasmids expressing Myc-HIPK2 alone and with both plasmids encoding Myc-HIPK2 and GFP-SENP1. As previously described, HIPK2 was localized to the discrete dot structure [10], and GFP-SENP1 was predominantly localized to the cytoplasm (Fig. 3, arrowhead). However, the GFP-SENP1 was translocated into the nucleus upon HIPK2 co-expression. In addition, HIPK2 exhibited a diffused localization pattern in the nucleoplasm with the relocalization of SENP1 (Fig. 3, arrow). HIPK2 desumoylation as the result of SENP1's activity

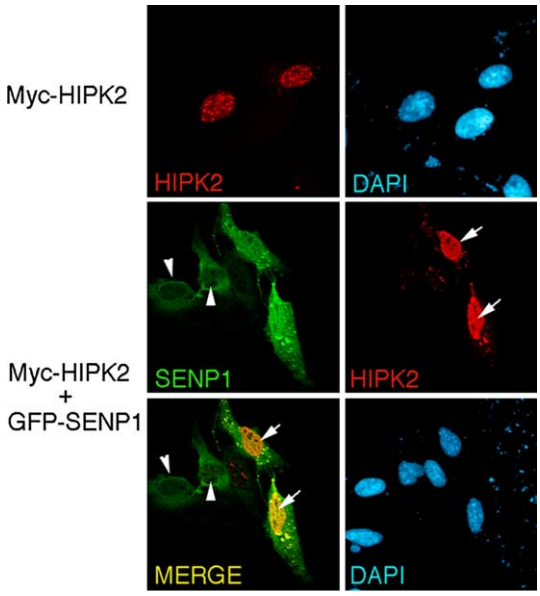

Fig. 3. Nuclear diffusion of HIPK2 upon the relocalization of SENP1. CV-1 cells were transfected with plasmids encoding Myc-HIPK2 alone or along with plasmids encoding GFP-SENP1. The transfected cells were fixed and stained with anti-Myc antibody. Myc-HIPK2 was visualized by the incubation of the cells with rhodamine-conjugated anti-mouse IgG. The nuclei of the cells were decorated with DAPI staining. The arrowhead and arrow indicate the cell expressing SENP1 alone and the cell expressing both SENP1 and HIPK2, respectively.

was evaluated with Western blot analysis following cotransfection with increasing amounts of SENP1 plasmids, along with Myc-HIPK2 and GFP-SUMO-1 expression plasmids. The expressed SENP1 was noted to deconjugate the SUMO-1 from the sumoylated HIPK2 in direct proportion to the amounts of SENP1 expressed (Fig. 4A). In order to explore these observations in further detail, we conducted two experiments, involving the in vitro desumoylation of HIPK2 and desumoylation using the catalytic mutant SENP1(C603A) in cultured cells. The affinity-purified GST-SENP1 was shown to effectively remove SUMO-1 moiety, in the presence of Ca^{2+} rather than Mn^{2+}, from the sumoylated HIPK2 which had been prepared via the immunoprecipitation of lysates using anti-Myc antibody, from cells transfected with Myc-HIPK2 and GFP-SUMO-1 (Fig. 4B). Our findings in this regard indicated that the desumoylation of HIPK2-SUMO conjugates was induced as the result of the direct catalytic activity of SENP1. In order to confirm the SENP1-specific desumoylation of HIPK2-SUMO conjugates in cultured cells, plasmids encoding wild-type His-SENP1 and the C603A mutant were transfected into CV-1 cells, in combination with plasmids expressing Myc-HIPK2 and GFP-SUMO-1. The cell lysates were then subjected to Western blotting using anti-GFP, anti-His and anti-Myc antibodies, in an attempt to detect cellular SUMO-conjugates, His-SENP1 and Myc-HIPK2, respectively. The wild-type SENP1 was determined to efficiently desumoylate cellular SUMO-conjugates, including the sumoylated HIPK2. However, the catalytically inactive mutant, SENP1(C603A), was determined to be unable to desumoylate HIPK2-SUMO conjugates and the cellular sumoylated proteins, and also exhibited a dominant negative effect antagonizing the functions of endogenous SENP1, in which the SUMO-conjugation of HIPK2 was comparably increased (Fig. 4C, lane 6). These

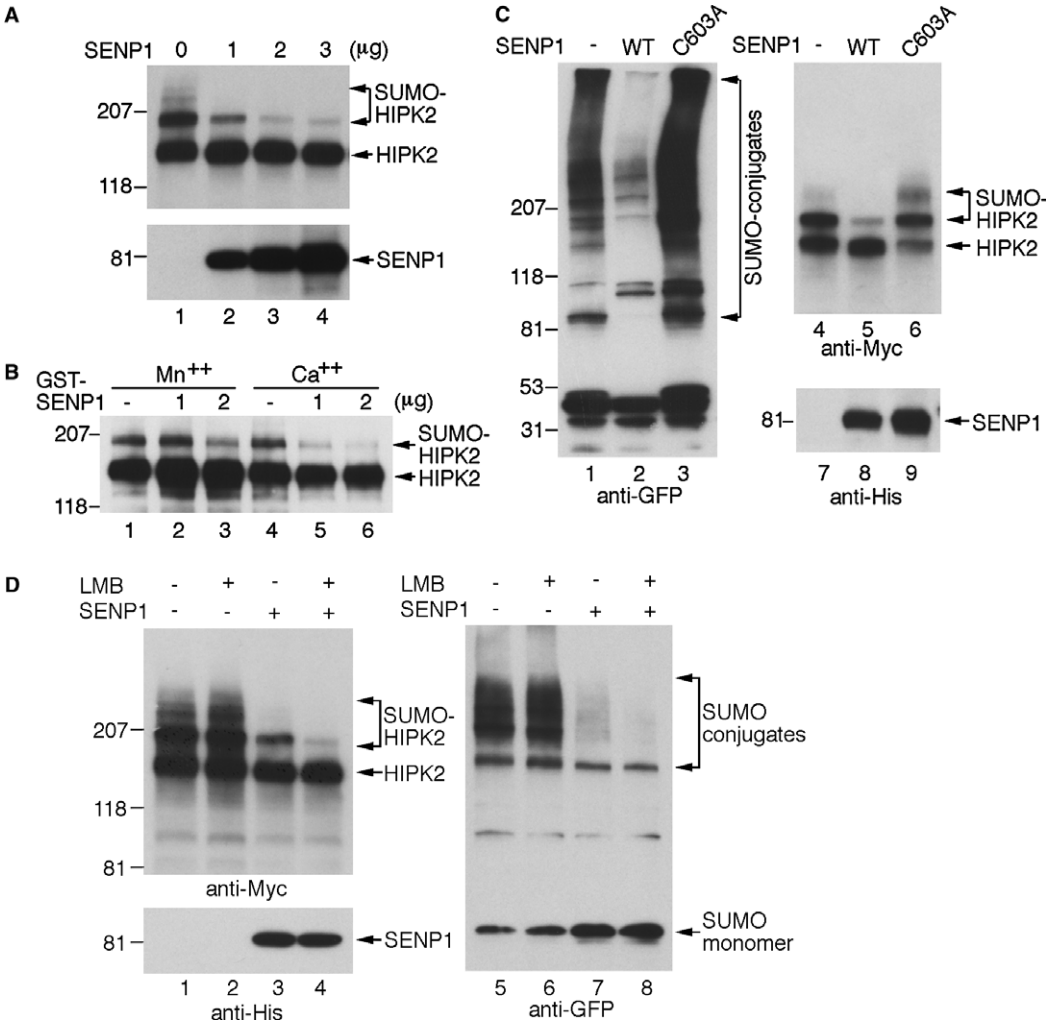

Fig. 4. Desumoylation of HIPK2-SUMO conjugates by SENP1. (A) Increasing amounts of the His-SENP1 plasmids were cotransfected into CV-1 cells together with the Myc-HIPK2 and GFP-SUMO-1 expression plasmids. The sumoylated HIPK2 were visualized with Western blotting using anti-Myc antibodies. (B) The sumoylated HIPK2 were immunoprecipitated from the lysates of cells transfected with the Myc-HIPK2 and GFP-SUMO-1 plasmids, and were subsequently used for in vitro desumoylation reactions in the presence of affinity-purified GST-SENP1, with Mn^{2+} or Ca^{2+} as a cofactor. (C) The wild-type SENP1 and C603A mutant plasmids were transfected into CV-1 cells, together with Myc-HIPK2 and GFP-SUMO-1. Both the sumoylated HIPK2 and the expressed wild-type or mutant SENP1 were detected with Western blotting using anti-Myc and anti-His antibodies, respectively (right panel). The total cellular SUMO-conjugates were visualized with anti-GFP antibodies (left panel). (D) CV-1 cells were transfected with Myc-HIPK2 and GFP-SUMO-1 plasmids along with or without His-SENP1 plasmids. Twenty-four hours after transfection, cells were treated with LMB for 6 h before Western blotting using anti-Myc antibodies (left panel). The total SUMO conjugates in the cells were detected with anti-GFP antibodies (right panel).

findings clearly indicate that SENP1 directly catalyzes the desumoylation of the HIPK2-SUMO conjugates. Taken together, SENP1 can act as a desumoylation enzyme for HIPK2-SUMO conjugates, both in vitro and in vivo. As sumoylations are nuclear events and most SUMO-conjugates are located within the nucleus, it appears that SENP1 might find its cellular targets subsequent to its nuclear relocalization. Therefore, we assessed the desumoylation of both HIPK2 and its cellular targets subsequent to the administration of LMB treatment. Indeed, as shown in Fig. 4D, LMB treatment enhanced the removal of SUMO-1 moiety from both HIPK2-SUMO-1 conjugates and the cellular proteins-SUMO-1 conjugates. This finding is consistent with the increased desumoylation of HIPK2-SUMO conjugates by SENP1 NES-M mutant (Fig. 2C, lane 5). Taken together, we conclude that HIPK2-SUMO conjugates are efficiently desumoylated as the result of SENP1 relocalization.

4. Discussion

We and others have previously demonstrated that HIPK2 is recruited into the PML body, during which the sumoylation of both PML and other nuclear body (NB) components appears to be vitally important [10,13]. The assembly and disassembly of PML bodies is known to be an extremely dynamic process, which is dependent upon a host of signaling cues and cellular stresses, including heat shock and Cadmium [31,32]. In this paper, we have shown that HIPK2-SUMO conjugates are the direct target of SENP1, and also that SENP1 itself is capable of shuttling between the cytoplasm and the nucleus. Bailey and O'Hare [22] reported that the catalytic mutant SENP1-(C603A) was localized to the nucleus and was recruited into NB upon the co-expression of PML. The PML-SUMO conjugates were also determined to be the target of SENP1 [18].

Our findings suggest that HIPK2-SUMO and PML-SUMO conjugates form a complex with SENP1 in the NBs, and that the balance between sumoylation and desumoylation is vitally important with regard to the dynamics of NBs. In support of this notion, we determined that HIPK2 was released from NBs to a diffused nucleoplasmic localization via the co-expression of SENP1 (Fig. 3). In addition, SENP1(C603A) was determined to function as a dominant negative mutant, and resulted in an enhancement of HIPK2 sumoylation (Fig. 4C). These data, when combined with our observations of the desumoylation of HIPK2-SUMO conjugates, both in vitro and in vivo, compelled us to conclude that SENP1 is responsible for the deconjugation of SUMO-1 from the sumoylated HIPK2. We also observed that the SUMO-1 moiety was removed from the sumoylated HIPK2 in the cultured cells by the activity of SuPr-1 and Axam-2, although this took place to a lesser extent (data not shown), which is consistent with a very recent study [33]. As the case of PML, therefore, it is conceivable that more than two SUMO-specific proteases might serve to regulate HIPK2 desumoylation.

To the best of our knowledge, this paper is the first to demonstrate that SENP1 is exported to the cytoplasm in a CRM1- and C-terminal NES-dependent manner. As shown in Fig. 1B, GFP-SENP1 was localized in the cytoplasm but relocalized into the nucleus after the application of LMB treatment, indicating the nucleo-cytoplasmic shuttling of SENP1. Amino acid sequence analysis also indicates that there may be three potential NLSs (KKTGRR$^{172–177}$, KKRK$^{574–577}$ and PYFRKRM$^{628–634}$) within SENP1. Among them, KKTGRR (aa 172–177) motif was analyzed previously [22]. In our study, the ΔC2 SENP1 mutant, which lacked both KKRK (aa 574–577) and PYFRKRM (aa 628–634), was shown to be localized in both the nucleus and the cytoplasm, and the ΔC1 SENP1 mutant, which lacked both NES and PYFRKRM (aa 628–634), was determined to be localized predominantly in the nucleus (Fig. 2B). These results suggest that each NLS alone is insufficient for the nuclear localization of SENP1, and that the two or three NLSs must work in concert to ensure the nuclear localization of SENP1. These explanations are also concordant with the observation that the KKTGRR$^{172–177}$ motif alone was insufficient for the localization of pyruvate kinase into the nucleus [22]. The C-terminal region of SENP1 is composed of the catalytic core domain of SENP1, containing two NLSs and one NES. SENP1 localization was also apparently influenced by the catalytic activity of SENP1. The SENP1-(C603A) mutant was shown to be localized within the nucleus (Fig. 2B), and evidently relocated to the dot structure upon the co-expression of PML or HDAC4 [22]. These findings showed that the catalytic activity of SENP1 and its interaction with binding partners are factors which help to determine the localization of SENP1. Although SENP1(C603A) mutant shows additional band that probably generated by sumoylation, it is not clear whether the sumoylation of SENP1 is involved in nuclear-cytoplasmic shuttling of SENP1. SENP1 localization could clearly be changed as the result of shifts in the balance between nuclear import and export, as well as of molecular interactions with targets. Consistent with this idea, co-expression of adenoviral E1A, which was shown to cooperate with E1B, which is sumoylated, for cellular transformation [34], induced the nuclear localization of SENP1 (data not shown). Probably, SENP1 localization might be controlled by the extracellular signaling pathway [35], and that concurrent post-translational modifications such as sumoylation or phosphorylation might add the fine control and complexity to the regulation of SENP1.

The NES motif of SENP1 identified in this study appears to be quite well-conserved among the other members of the SENP family, including SENP2/Axam, SuPr-1, SENP3 and SENP5 (Fig. 2D). Interestingly, cytoplasmic SENP proteins such as SUSP1/SENP6 and SUSP2/SENP7 [23] contain no conserved NES within their C-terminal regions, but all nuclear SENP proteins harbor the NES motif, although they exhibit different localizations, in either the nuclear membrane, the PML body, or the nucleolus [15,36]. This raises a possibility that all nuclear SENP proteins might be exported depending on the cellular contexts and existence of environmental stimuli, as the case of SENP1. Further studies might provide more insight into the relevance of the conservation of the NES motif among members of SENP family.

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